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(54) Title: HEPATOBILIARY NMR CONTRAST AGENTS

(57) Abstract

A method of decreasing the NMR relaxation times (T_1 or T_2) of water protons in contact with a biological tissue, the method involving administering to a human patient an NMR contrast agent comprising a paramagnetic ion complexed with a chelating substance, the contrast agent being characterized in that it is capable of binding non-covalently and non-immunologically to a component of the tissue, and as a result of such binding is capable of enhancing relaxivity of the water protons by a factor of at least 2, compared to the relaxivity induced by the paramagnetic substance alone free in solution, and subjecting the patient to NMR imaging.

HEPATOBILIARY NMR CONTRAST AGENTS

Background of the Invention

This invention relates to diagnostic NMR imaging.

NMR imaging has been used in medical diagnosis
for a number of years. The use of contrast agents to
enhance its diagnostic utility has only recently
appeared. For example, Gries et al. German Patent DE
3,129,906 describes NMR contrast agents which consist of
a paramagnetic ion complexed with a chelating agent and
a base or acid, e.g., the di-N-methylglucosamine salt of
manganese chelated with EDTA.

Summary of the Invention

The present invention provides an in vivo
method of decreasing the NMR relaxation times of water
protons in contact with a biological tissue. The method
involves administering to a human patient an NMR
contrast agent containing a paramagnetic metal ion
complexed with a chelating substance, the contrast agent
being characterized in that it is capable of binding
non-covalently and non-immunologically to a component of
the tissue, and as a result of such binding is capable
of enhancing the relaxivity (i.e., decreasing the NMR
relaxation times T_1 or T_2) of the water protons by a
factor of at least 2, compared to the relaxivity induced
in such water protons by the paramagnetic substance
alone free in solution; and subjecting the patient to
NMR imaging.

Preferably, the contrast agent has a specific
affinity for the biological tissue in which binding
occurs. (As used herein, "specific affinity" means
capable of being taken up by, retained by, or bound to a
particular tissue or tissue component to a substantially
greater degree than other tissue or tissue components;

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agents which have this property are said to be
"targeted" to the "target" tissue or component.)

The components to which the agents of the
invention bind are generally particular chemical

5   classes, e.g., proteins, lipids, or polysaccharides. It
has been found that the tight binding of the agents to
these components causes an increase (at least by a
factor of 2) in the longitudinal $(1/T_1)$ and transverse
$(1/T_2)$ relaxivity of water protons by the metal

10   complex. Relaxivity enhancement is apparently due in
large part to an alteration in the effective correlation
time of the electron-nuclear interaction, as described
in Lauffer et al. (1985) Magn. Res. Imaging $\underline{3}$, 11.

In the agents of the invention, the toxic

15   paramagnetic ion (e.g., gadolinium) is strongly
complexed by a chelating agent to reduce toxicity; it
has been found that such agents are effective in
reducing T_1 and T_2 (discussed below), despite the
relatively lower accessibility of the paramagnetic ion

20   to the surrounding water protons.

Examples of classes of chelating substances of
the invention are porphyrins, cryptate compounds, and
bis, tris, or tetra-catechol compounds.

The contrast agents of the invention which bind

25   tightly to proteins are also taken up specifically by
human hepatocytes, compared to human reticuloendothelial
cells, and, because hepatocytes make up the bulk of the
liver, provide superior NMR imaging of the liver. The
agents thus allow visualization of hepatocarcinoma or

30   metastatic tumors of the liver, whose cells take up the
agents at a different rate, or retain the agent for a
different length of time, than normally functioning
hepatocytes. The invention also allows the use of NMR

imaging to monitor liver function, as manifested by
uptake or retention rates of the contrast agents of the
invention.

Other features and advantages of the invention

5   will be apparent from the following description of the
preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

Preferred embodiments of the invention are
described below.

10   Properties of Contrast Agents

Many agents of the invention will have utility
in a wide range of applications, because the chemical
requirements for tight binding to many components are
the same, and also because in some instances the same

15   properties which induce tight binding also influence
tissue specificity. For example, the properties of
agents which cause selective uptake by hepatocytes
compared to reticuloendothelial cells also cause tight
binding of the agents to proteins, e.g., intracellular

20   proteins of hepatocytes.

The preferred NMR contrast agents of the
invention possess a number of physical/chemical
properties, discussed below, related to their utility in
diagnostic applications.

25   In order for agents which are targeted to
provide the NMR contrast needed for imaging, they must
alter the proton NMR relaxation time in the target
component. Thus the agents must have properties which
cause them to selectively be taken up by or bound to the

30   target. This is achieved either by means of a higher
rate of uptake of the contrast agent by the target, or
by a different retention profile between target and
non-target tissues. NMR contrast is achieved by the

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altering, by the paramagnetic portion of the agent, of T_1 (longitudinal relaxation time) or T_2 (transverse relaxation time) of the water protons in the target.

As mentioned above, one tissue component to
5   which the agents of the invention can bind are
proteins. These can be intracellular proteins, e.g.,
the proteins such as ligandin (also known as Y protein
or glutathione-S-transferase (EC 2.5.1.18) and Protein A
(also known as Z protein or fatty acid binding protein)
10   inside hepatocytes (J. Clin. Invest. 48, 2156-2167
(1969)). Where the agents are targeted to particular
cells such as hepatocytes, it is generally the cells,
and not the intracellular proteins themselves, to which
the agents are targeted as a result of the properties of
15   the agents, which properties in turn cause tight binding
to the intracellular proteins of those cells.

Agents which have protein-binding properties
can bind not only to intracellular proteins but also to
serum proteins such as human serum albumin (HSA). This
20   binding provides selective enhancement of intravascular
structures or patterns on NMR images, permitting
diagnosis of blood/brain barrier disruptions caused,
e.g., by strokes and brain tumors, and also permitting
flow imaging of the blood. For example, some agents can
25   bind to both HSA and ligandin in vivo, and thus
represent dual intravascular-hepatobiliary agents.

Another important protein which is bound
tightly by the protein-binding agents is the immature,
poorly cross-linked collagen present in tumors. This
30   collagen can be bound tightly by NMR contrast agents
which comprise a paramagnetic metal ion complexed with a

porphyrin. When these proteins are bound, the agent
serves the dual roles of tumor targeting and relaxivity
enhancement.

Protein binding is provided for by the
incorporation of hydrophobic groups into the agent, and
providing the agent with the proper net charge.

Hydrophobic Binding

Binding is promoted when both the contrast
agent and the protein contain one or more hydrophobic
domains; the contrast agent binds non-covalently to the
protein through Van der Waals interactions between the
hydrophobic domains, thus enhancing binding.

Where the target is a protein, lipophilicity
enhances binding of the contrast agents to the protein.
Lipophilicity is provided by a non-polar structure, the
presence of at least one aryl group (e.g., a substituted
or unsubstituted phenyl ring), at least one halogen
atom, and/or hydrophobic alkyl groups. For
lipophilicity, it is also desirable that the contrast
agent not carry excessive charge, i.e., of absolute
value greater than 4, at physiological pH.

Lipophilicity is expressed in terms of
octanol:water coefficient, determined by introducing a
small amount (0.1 mM) of the radiolabeled contrast
agent into equal volumes of octanol and Tris buffer (50
mM, pH 7.4). The coefficient of the agents of the
invention is preferably at least 0.005, and more
preferably at least 0.01.

Another index related to lipophilicity is that
of protein-binding. Binding capacity can be expressed
as the percentage of the agent bound to 4.5% human serum
albumin (HSA) at a concentration of 0.2 mM of the agent,
as determined by equilibrium dialysis. For

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protein-targeted agents, preferably at least 15%, and
more preferably at least 50%, of the agent, binds to HSA.

Electrostatic Interactions

Binding may be further increased if
5 electrostatic interactions between the contrast agent
and protein are possible. Thus, if the protein is known
to have positively charged binding sites (e.g., human
serum albumin) or if the protein is known to have the
highest affinity for anionic ligands (e.g., albumin,
10 ligandin or Protein A), then the net charge on the agent
should be negative, preferably -1 to -4. Also, direct
electrostatic interactions with positively charged
residues may be promoted if the agent has additional
negatively charged groups (e.g., sulfonate or
15 carboxylate) that are not coordinated to the metal ion
in solution.

Alternatively, if the binding sites are known
to have anionic character, the agent should have overall
positive charge.

20   Molecular Weight

The agents preferably have a molecular weight
of at least 250, and more preferably over 300.

Solubility

To facilitate administration and uptake, the
25 agents should have good water solubility, and preferably
should be soluble to a concentration of at least 1.0 mM
in normal saline at $20^{o}C$.

Relaxivity

The contrast agents of the invention must, as
30 mentioned above, lower either T_1 or T_2 or both. The
ability to achieve this is referred to as "relaxivity."

Relaxivity is optimal where the paramagnetic
ion, when bound to the chelating ligand, still has one

or more open coordination sites for water exchange.
Generally, one or two such sites are preferred, since
the presence of more than two open sites in general will
unacceptably increase toxicity by release of the metal
ion in vivo. However, zero open coordination sites may
also be satisfactory, though not preferable, since
second coordination sphere water molecules are still
relaxed and binding-enhancement is still possible.

In vitro relaxivity is expressed in units of
s^{-1} mM^{-1}, or change in $1/T_1$ or $1/T_2$ per mM
agent, as measured in saline at 20 MHz. Preferably the
agents have an in vitro relaxivity of at least 0.5 s^{-1}
mM^{-1}, more preferably at least 1.0 s^{-1} mM^{-1}.

Relaxivity can also be measured in vivo for the
tissue component of interest. In vivo relaxivity is
expressed in units of s^{-1} (mmol/gram of tissue)$^{-1}$,
representing the change in $1/T_1$ or $1/T_2$ above that
of saline-injected controls caused by the agents,
divided by the concentration of the agent (in
mmol/gram of tissue). Tissue concentration is
measured using agents made with radiolabeled
paramagnetic ions. Preferably, the in vivo relaxivity
of the agents in liver tissue is at least 1.0 s^{-1}
(mmol/g)$^{-1}$. The agents should bind sufficiently
tightly to enhance relaxivity by a factor of at least
2. This increased relaxivity will allow for lower doses
of the contrast agents and thus a higher margin of
safety in their use.

To maximize the degree of relaxivity
enhancement, it is desirable to maximize the rigidity of
the binding interaction. Preferably, this is achieved
by providing the contrast agent with at least one aryl
or aliphatic group which makes multiple contacts with

the biological binding site, preventing free rotation.
Additionally, free (non-coordinating) charged groups
(e.g., sulfonate or carboxylate) can be incorporated
into the agent to promote electrostatic interactions
5   with positively charged amino acid residues; this will
increase both the binding affinity and rigidity.

A different strategy to increase the relaxivity
of metal complexes is to alter the configuration of the
donor atoms around the metal ions to achieve the most
10 symmetrical orientation. This symmetry of the ligand
field may lead to longer electron spin relaxation times,
and higher relaxivities. The DOTA ligands for Gd^{+3}
(described below) are an example in which the symmetry
is very high (almost cubic) compared to, e.g.,
15 DTPA-derived ligands (described below), which wrap
around the metal ion in an anisotropic fashion. An
additional benefit of symmetry-constrained macrocyclic
ligands like DOTA is their high kinetic stability (<u>vide</u>
<u>infra</u>).

20   Toxicity

The contrast agents must have acceptably low
toxicity levels at the dosage required for contrast
enhancement, and preferably have an LD_{50} of at least
0.05 mmol/kg. Toxicity of the contrast agents is a
25 function of both the inherent toxicity of the intact
complex, and of the degree to which the metal ion
dissociates from the chelating agent; toxicity generally
increases with the degree of dissociation. For
complexes in which kinetic stability is low, a high
30 thermodynamic stability (a formation constant of at
least 10^{15} M $^{-1}$, and more preferably at least 10^{20}
M^{-1}) is desirable to minimize dissociation and its
attendant toxicity. For complexes in which kinetic

stability is comparitively higher, dissociation can be minimized with a lower formation constant, i.e., 10^{10} M^{-1} or higher. Kinetically stable complexes generally contain a paramagnetic metal ion, e.g., gadolinium

5   (III), complexed with a highly constrictive chelating agent, e.g., dibenzo-1, 4, 7, 10-tetraazacyclotetradecene 1, 4, 7, 10-tetraacetic acid (dibenzo-DOTA).

       Toxicity is also a function of the number of
10   open coordination sites in the complex; the fewer open coordination sites, the less tendency there is, generally, for the chelating agent to release the cytotoxic paramagnetic ion. Preferably, therefore, the complex contains two, one, or zero open coordination
15   sites. The presence of one or even two open coordination sites can be acceptable in agents in which the paramagnetic substance has a high magnetic moment (i.e., is strongly paramagnetic), and can thus affect T_1 or T_2 at a low dosage; an example is gadolinium,
20   which is strongly paramagnetic owing to its seven unpaired electrons.

       The paramagnetic portion of the contrast agents of the invention can be any paramagnetic ion of the transition metal or lanthanide series which has at least
25   one, and more preferably five or more, unpaired electrons, and a magnetic moment of at least 1.7 Bohr magneton. Suitable ions include gadolinium (III), iron (III), manganese (II and III), chromium (III), copper (II), dysprosium (III), terbium (III), holmium (III),
30   erbium (III), and europium (III); most preferred are gadolinium (III), and iron (III), and manganese (II).

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Chelating Ligand

The following discussion applies to chelating ligands which cause the agents of the invention to bind tightly to proteins and to be selectively taken up by functioning hepatocytes.

The organic chelating ligand should be physiologically compatible and preferably contains at least 1 aryl ring which may be substituted with halogen atoms and/or C_1-C_{10} alkyl groups. The molecular size of the chelating ligand should be compatible with the size of the paramagnetic substance. Thus gadolinium (III), which has a crystal ionic radius of 0.938Å, requires a larger chelating ligand than iron (III), which has a crystal ionic radius of 0.64Å. Preferably, the chelating ligand is a single multidentate ligand. Such ligands maximize the stability of the contrast agents towards hydrolysis, and minimize the transfer of the metal ion from the contrast agent to binding sites on the target component.
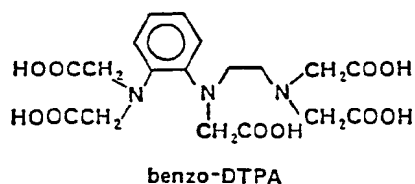
One suitable class of chelating ligands are ethylenebis-(2-hydroxyphenylglycine) ("EHPG"), and derivatives thereof, including 5-Cl-EHPG; 5-Br-EHPG; 5-Me-EHPG; 5-t-Bu-EHPG; and 5-sec-Bu-EHPG. EHPG and derivatives thereof have the structure:


R= Cl,Br,I,CH₃,t-Bu,sec-Bu
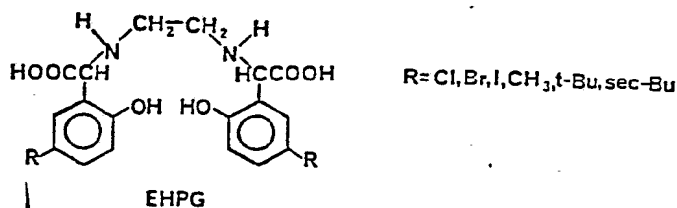
EHPG

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Although substitution at the 5 position of EHPG is the most effective in increasing lipophilicity, substitution at any position on the two phenyl rings can be used.

5   Another suitable class of chelating ligands are benzodiethylenetriamine-pentaacetic acid (benzo-DTPA) and derivatives thereof, including dibenzo-DTPA; phenyl-DTPA; diphenyl-DTPA; benzyl-DTPA; and dibenzyl-DTPA. Two of these compounds have the

10   structures shown below:

benzo-DTPA   dibenzo-DTPA
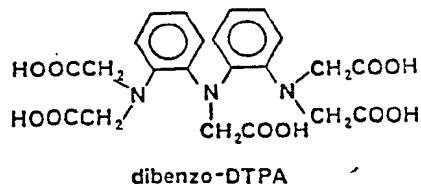
Another class of suitable chelating ligands are bis-2 (hydroxybenzyl)-ethylene-diaminediacetic acid (HBED) and derivatives thereof. The structure of HBED is shown below:
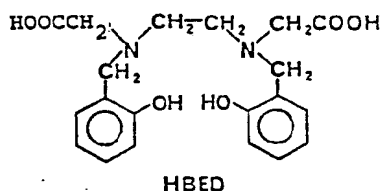
HBED

The HBED ligand advantageously has a very high formation constant for iron of 10^{40}. This ligand is available from the Strem Chemical Company.

Another suitable class of chelating ligands is the class of macrocyclic compounds which contain at least 3 carbon atoms, more preferably at least 6, and at least two hetero (O and/or N) atoms. The macrocyclic compounds can consist of one ring, or two or three rings joined together at the hetero ring elements. One suitable class of mono-macrocyclic chelating ligands has the general formula
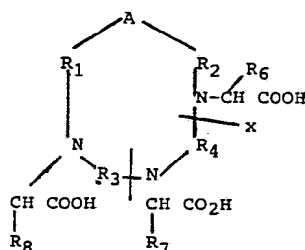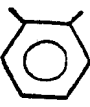
where A is -N- or , X is 0 or 1, each R_5, R_6,
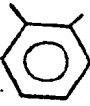
R_5-CH$_2$CO$_2$H

R_7, and R_8, independently, is H or methyl, and each, R_1, R_2, R_3, and R_4, independently, is ethyl, propyl, butyl, pentyl, or , provided that when A is   -N-   ,

R_5-CH$_2$CO$_2$H

at least one R group must be . The aryl groups may be substituted with halogen atoms or C_1-C_4 alkyl groups. Examples of suitable macrocyclic ligands include benzo-DOTA, where DOTA is 1, 4, 7, 10-tetraazacyclotetradecane-1, 4, 7, 10-tetraacetic acid; dibenzo-DOTA; benzo-NOTA, where NOTA is 1, 4, 7-triazacyclononane- N, N', N''-triacetic acid; benzo-TETA, where TETA is 1, 4, 8, 11-tetraazacyclotetradecane-1, 4, 8, 11-tetraacetic acid; benzo-DOTMA, where DOTMA is 1, 4, 7, 10-tetraazacyclotetradecane-1, 4, 7, 10-tetra(methyl tetraacetic acid); and benzo-TETMA, where TETMA is 1, 4, 8, 11-tetraazacyclotetradecane-1, 4, 8, 11-(methyl tetraacetic acid).

Hydrophobicity, and thus lipophilicity, can also be provided, in the case of ligands (e.g., DOTA derivatives) containing ethylenediamine portions by attaching the above hydrophobic substituents directly to the ethylene carbon atoms. For example, DOTA has the structure:
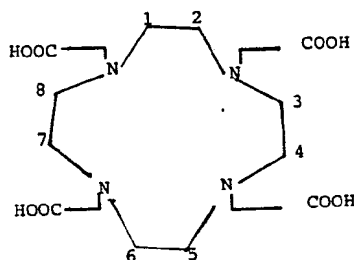
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Hydrophobic substituents, e.g., fused phenyl rings or
C_{1-5} alkyl groups, can be attached to one or more of
carbon atoms 1-8 of DOTA.

     Another suitable class of chelating ligands are
DTPA derivatives containing hydrophobic substituents.
Structures of suitable such derivatives are given below,
in which each R_1, R_2, R_3, R_4 and R_5,
independently, can be a C_{6-10} aryl group, e.g., phenyl
or benzyl; or a C_{1-5} aliphatic group, e.g., methyl or
ethyl.
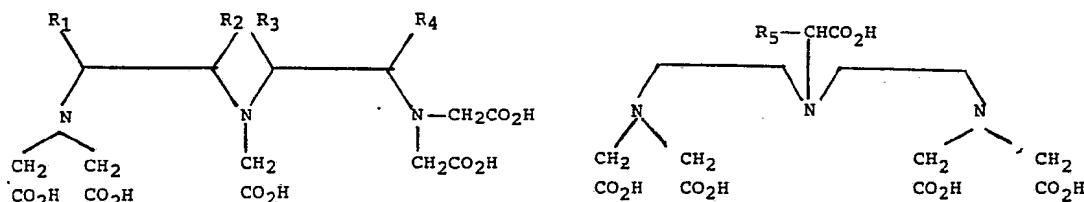
     Another suitable class of chelating ligands are
derivatives of 1,3-propylenediaminetetraacetic acid
(PDTA) and triethylenetetraaminehexaacetic acid (TTHA),
given below. Each R_1, R_2, R_3, R_4, R_5, R_6,
and R_7 group, independently, can be a C_{6-10} aryl
group, e.g., phenyl or benzyl; or a C_{1-5} aliphatic
group, e.g., methyl or ethyl.

PDTA
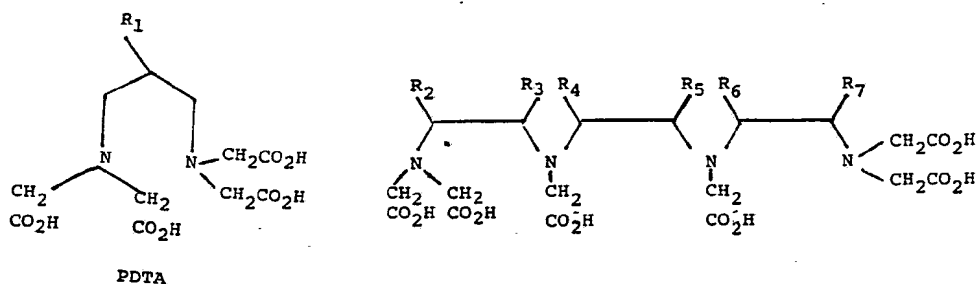
- 15 -

Another suitable class of chelating ligands are derivatives of 1,5,10-N,N^1,N^{11}-tris(2,3-dihydroxybenzoyl)-tricatecholate (LICAM) and 1,3,5-N,N',N''-tris(2,3-dihydroxybenzoyl)aminomethylbenzene (MECAM), having the structures given below. Each R_1, R_2, R_3, R_4, R_5, and R_6, independently, can be CO_2H, SO_3H, H, a halogen, e.g., Cl, or a C_{1-5} alkyl group, e.g., methyl or ethyl.


LICAM

MECAM
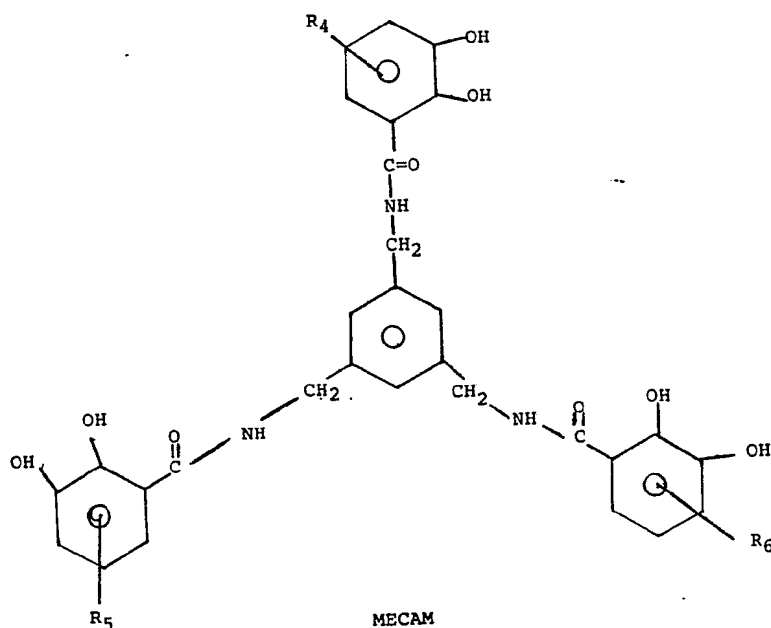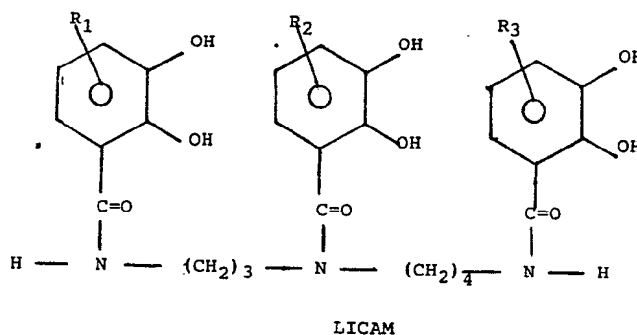
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Synthesis

        The contrast agents of the invention can be
synthesized from commercially available or readily
synthesized reagents using conventional synthetic
methods. In general, a salt of the paramagnetic ion is
added to a slightly alkaline (pH 7.4-9) aqueous solution
of the chelating ligand and the resulting mixture is
stirred for 3-24 hours at room temperature. The
resulting contrast agent is then used immediately or
stored in lyophilized form or in physiological buffer
until use.

        The synthesis of iron (III)-(EHPG)$^-$ is
carried out as follows. EHPG (Sigma) is dissolved at
room temperature in distilled, deionized water
maintained at pH 8-9 by addition of 1M NaOH. Solid
$FeCl_3 \cdot 6H_2O$ is added to the solution and the pH
adjusted to 7.4 with 1M NaOH. The resulting dark red
solution is then stirred at room temperature for 30
minutes, after which it is filtered with 0.2 mm
micropore filters (Gelman). The concentration of iron
(III)-(EHPG)$^-$ is determined by visible absorption of
diluted aliquots using a Beckman Spectrophotometer and
an extinction coefficient at 480 nm of 4300 $CM^{-1}M^{-1}$.

        To make iron chelates of EHPG derivatives the
first step is to make the appropriate EHPG derivative,
according to Mannich reaction, described in Theodorakis
et al. (1980) J. Pharm. Sci 69, 581; the reaction
employs ethylenediamine, dichloroacetic acid, and the
appropriate parasubstituted phenol. The reaction scheme
for 5-Br-EHPG is:
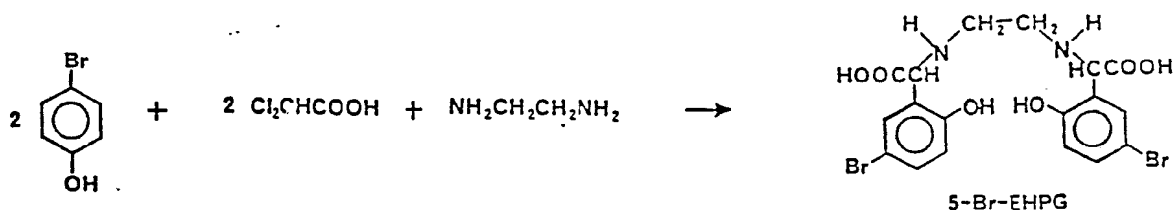
5-Br-EHPG

Iron (III)-(5-Cl-EHPG)⁻, iron
(III)-(5-Bu-EHPG)⁻, iron (III)-(5-Me-EHPG)⁻, and
iron (III)-HBED are prepared in analogous fashion to
iron-EHPG.

5   The structure of iron-EHPG is:
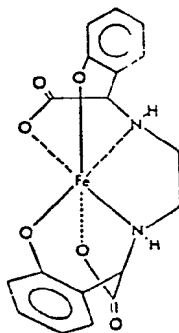
The octanol/water partition coefficients and
HSA binding percentages of Iron-EHPG, Iron-(5-Br-EHPG),
and Iron (HBED) are shown below:

	$\dfrac{[octanol]}{[water]}$	% bound to HSA
Iron-EHPG	0.013	17
Iron-(5-Br-EHPG)	0.89	82
Iron-HBED	0.050	34

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The macrocyclic DOTA chelating ligands are
synthesized generally as described in Desreux et al.
(1984) Inorg. Chem. 19, 1319, generally according to the
reaction
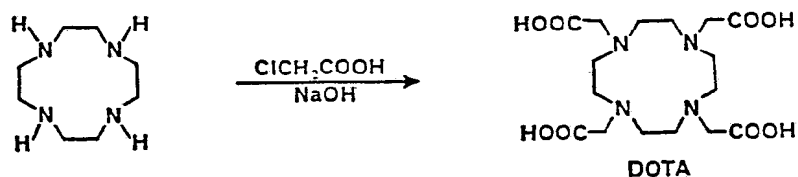
DOTA itself lacks sufficient lipophilic groups
15   for hepatocellular uptake. Two derivatives with the
required lipophilicity (provided by fused phenyl rings),
benzo-DOTA and dibenzo-DOTA, are made according to the
following general reaction scheme. (Alternatively,
hydrophobic substituents can be incorporated into, e.g.,
20   DOTA, via substituted ethylenediamines prepared
according to Meares et al. (Anal. Biochem. 100 152-159
(1979).)
     DTPA derivatives (e.g., benzo-DTPA and
dibenzo-DTPA) are made by methods analogous to the
25   methods used for making benzo-EDTA (McCandlish et al.
(1978) Inorg. Chem. 17, 1383).
     Paramagnetic ion chelating ligand complexes
made using DOTA derivatives are made generally as
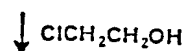described earlier, with a longer time (24 hours) and
30   higher reaction temperatures being required for the
formation of metal ion/macrocyclic ligand complexes. A
reaction scheme is shown below:

Ts = CH₃—⟨benzene⟩—SO₂—
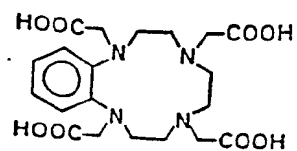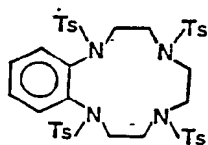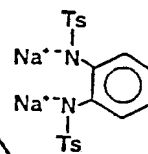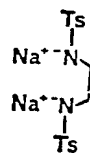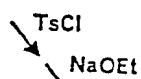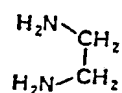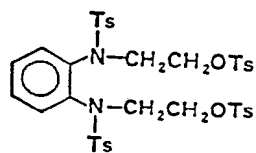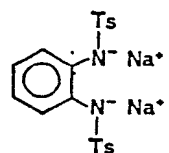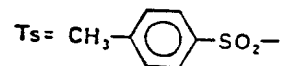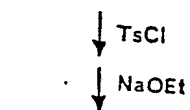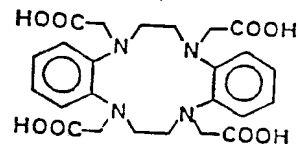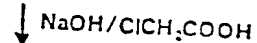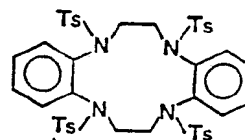
benzo-DOTA

dibenzo-DOTA

Use

The contrast agents of the invention are
administered orally or intravenously in physiological
buffer. Dosage depends on the sensitivity of the NMR
5   imaging instrumentation, as well as on the composition
of the contrast agent. For example, a contrast agent
containing a highly paramagnetic substance, e.g.,
gadolinium (III), generally requires a lower dosage than
a contrast agent containing a paramagnetic substance
10   with a lower magnetic moment, e.g., iron (III). In
general, dosage will be in the range of about .001-1
mmol/kg, more preferably about 0.005 - 0.05 mmol/kg.

Following administration of the contrast agent,
conventional NMR imaging is carried out; the choice of
15   pulse sequence (inversion recovery, IR; spin echo, SE)
and the values of the imaging parameters (echo time, TE;
inversion time, TI; repetition time, TR) will be
governed by the diagnostic information sought. In
general, if one desires to measure T_1, then TE should
20   be less than 30 milliseconds (or the minimum value) to
maximize T_1-weighting. Conversely, if one desires to
measure T_2, then TE should be greater than 30
milliseconds to minimize competing T_1 effects. TI and
TR will remain approximately the same for both T_1-
25   and T_2-weighted images; TI and TR are generally on
the order of about 200-600 and 100-1000 milliseconds,
respectively.

NMR Imaging Using Iron (III)-(EHPG)

Iron (III)-(EHPG)$^-$ was prepared as described
30   above and used for in vivo imaging of rat livers as
follows.

- 21 -

Fasted male Sprague-Dawley rats (of average
weight of about 400g) were anesthetized with
intraperitoneal pentobarbitol (50 mg/kg), placed on a
calibrated carrier, and subjected to NMR imaging, along
5   with calibration tubes containing paramagnetically-doped
water or agar gels of known T_1 and T_2, to establish
an initial baseline image. NMR imaging was performed
with a horizontal bore (8cm) superconducting magnet
system (Technicare Corp.) at a magnetic field strength
10 of 1.4 tesla (^1H resonance of 61.4 MHz). Images were
obtained using a 2-D Fournier transform technique with a
slice selection determined by selective irradiation.
All images were obtained using 128 phase encoded
gradient steps. To maximize T_1 contrast, an IR pulse
15 sequence was used (TE 15 msec, TI 400 msec, TR 1000
msec).

After baseline images were obtained, the rats
were removed from the magnet and injected in the tail
vein with 0.2 mmol/kg of iron (III)-(EHPG)$^-$. As a
20 comparison, some rats received 0.2 mmol/kg of iron
(III)-(DTPA)$^{-2}$ instead. The rats were then reinserted
into the magnet, along with the calibration tubes, in
the same position as for the initial baseline imaging.
Imaging began immediately and continued for 1.5-3
25 hours. Background-subtracted, region-of-interest
intensity values of liver and muscle were obtained for
each image; these values were then normalized for any
alteration in the signal intensity of the calibration
tubes.

30 The IR 1000/400/15 images of rats which
recieved iron (III)-(EHPG)$^-$ demonstrated a marked and
prolonged increase in signal intensity of the liver
consistent with a short T_1. In contrast, images of

rats which received iron $(III)-(DTPA)^{-2}$ demonstrated only small and transient increases in liver intensity. This is presumably because, unlike iron $(III)-(EHPG)^-$, iron $(III)-(DTPA)^{-2}$ distributes throughout the

5 extracellular liver space, rather than in functioning hepatocytes, and is rapidly excreted into the urine.

Ex vivo biodistribution studies measuring the T_1 and T_2 values of excised rat liver, blood, spleen, and thigh muscle at various post-injection times

10 also demonstrated that iron $(III)-(EHPG)^-$ is predominantly taken up by functioning hepatocytes, and thus decreases the relaxation times of water protons in these cells.

Rats given intravenous doses of 2.0 mmol/kg of

15 iron-EHPG suffered no apparent ill effects over a two-week observation period.

It is believed that the mechanism of operation of iron-EHPG is as follows. Relaxation time enhancement normally occurs where the unpaired electrons of the

20 paramagnetic substance interact with water molecules directly bound to the paramagnetic substance; the degree of enhancement is inversely related to the distance from the paramagnetic center to the water molecules. In iron (III)-(EHPG)-, however, there are no directly bound

25 water molecules. Relaxation time enhancement, therefore, probably results mainly from the interaction between the paramagnetic substance and indirectly bound, second coordination sphere water molecules. It is believed that since there are a sufficiently large

30 number of these outer-sphere water molecules, appreciable relaxation time enhancement occurs despite the larger distance between the water molecules and the paramagnetic substance.

Other embodiments are within the following claims.

<u>Claims</u>

1.   A method of decreasing the NMR relaxation
times (T_1 or T_2) of water protons in contact with a
biological tissue, said method comprising

administering to a human patient an NMR
contrast agent comprising a paramagnetic ion complexed
with a chelating substance, said contrast agent being
characterized in that it is capable of binding
non-covalently and non-immunologically to a component of
said tissue, and as a result of such binding is capable
of enhancing relaxivity of said water protons by a
factor of at least 2, compared to the relaxivity induced
by said paramagnetic substance alone free in solution,
and

subjecting said patient to NMR imaging.

2.   The method of claim 1 wherein said NMR
contrast agent has specific affinity for said biological
tissue.

3.   The method of claim 1 wherein said
component is a protein and said contrast agent and said
protein each contains one or more hydrophobic domains,
so that said contrast agent binds non-covalently to said
protein through Van der Waals binding interactions
between said hydrophobic domains.

4.   The method of claim 1 wherein a domain in
said component and said contrast agent are of opposite
charge, so that binding is promoted via electrostatic
interactions.

5.   The method of claim 1 wherein said contrast
agent binds to said component via multiple hydrogen
bonding interactions.

6. The method of claim 1 wherein said contrast agent binds to said component via more than one of the mechanisms of claims 3-5.

7. The method of claim 3 wherein said biological tissue is blood and said protein is human serum albumin.

8. The method of claim 3 wherein said biological tissue comprises human hepatocytes and said protein is the intracellular protein ligandin or protein A.

9. The method of claim 3 wherein said contrast agent binds to ligandin, Protein A, and HSA and is thus a dual intravascular/hepatobiliary NMR contrast agent.

10. The method of claim 3 wherein said protein is the immature, poorly cross-linked collagen in neoplastic tissue.

11. The method of claim 1 wherein said paramagnetic ion has at least two unpaired electrons.

12. The method of claim 1 wherein said chelating substance contains at least one aryl, aliphatic, non-coordinating sulfonate, or non-coordinating carboxylate group.

13. The method of claim 1 wherein said chelating substance comprises an aminocarboxylate derivative.

14. The method of claim 1 wherein said chelating substance comprises a porphyrin.

15. The method of claim 1 wherein said chelating substance comprises a cryptate compound.

16. The method of claim 1 wherein said chelating substance comprises a bis-, tris-, or tetra-catechol compound.

1   17. The method of claim 1 wherein said
2   component of said tissue comprises a protein in the
3   liver or bile duct of said patient, said agent being
4   further characterized in that it is taken up
5   preferentially by human hepatocytes, compared to human
6   reticuloendothelial cells.
7   18. The method of claim 1 wherein said agent
8   is further characterized in that it comprises a complex
9   of said paramagnetic substance and an organic chelating
10 ligand, said complex being characterized in that
11   it has a solubility to at least 1.0 mM
12 concentration in normal saline,
13   it has a molecular weight greater than 250, and
14   it has a charge of an absolute value of 2 or
15 less.
16   19. The method of claim 1 wherein said agent
17 is further characterized in that it contains at least
18 one aryl ring.
19   20. The method of claim 17 wherein said
20 complex is further characterized in that its
21 lipophilicity is sufficiently high to cause it to be
22 taken up in greater amount by normally functioning human
23 hepatocytes than by hepatocarcinoma cells.
24   21. The method of claim 1 wherein the formation
25 constant of said complex is at least $10^{15} M^{-1}$.
26   22. The method of claim 17 wherein said agent
27 is further characterized in that it exhibits an
28 octanol:water coefficient of at least 0.005.
29   23. The method of claim 17 wherein said agent
30 is further characterized in that it exhibits an
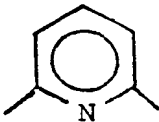31 octanol:water coefficient of at least 0.01.

1   24.   The method of claim 1 wherein said agent
2   is further characterized in that at least 15% of said
3   agent binds to 4.5% human serum albumin at a
4   concentration of 0.2 mM agent.
5   25.   The method of claim 24 wherein at least
6   50% of said agent binds to 4.5% human serum albumin at a
7   concentration of 0.2 mM agent.
8   26.   The method of claim 1 or 17 wherein said
9   macrocyclic compound has the formula
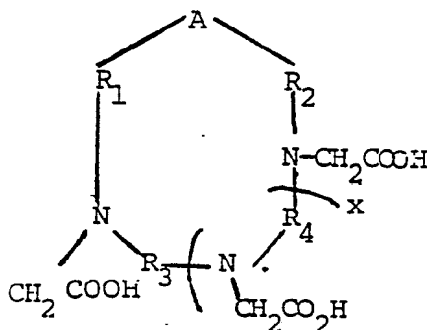
10   where A is -N-   or   , X is 0 or 1, and each
11   CH$_2$CO$_2$H
12   R$_1$, R$_2$, R$_3$, and R$_4$, independently, is ethyl,
13   propyl, or   , provided that when A is -N-   ,
14   CH$_2$CO$_2$H
15   at least one R group must be   .

INTERNATIONAL SEARCH REPORT

International Application No   **PCT/US86/01035**
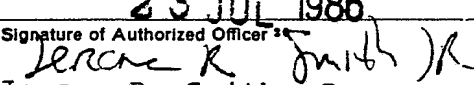
I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁹

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): A61B 6/00
US CL.: 128/654

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System	Classification Symbols
US	128/653,654 424/4,9 260/429J

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
Y	US,A, 4,308,249 (FRANK ET AL) 29 DEC. 1981 See the entire document	1-26
Y	US,A, 4,472,509 (GANSOW ET AL) 18 SEPT. 1984 See col. 3, lines 18-66	1,11,17-19, 24-26
A	US,A, 4,150,047 (COE ET AL) 17 APRIL 1979 See the entire document	
A	US,A, 4,352,751 (WIEDER ET AL) 05 OCT. 1982 See the entire document	
A	US,A, 4,331,647 (GOLDENBERG) 25 MAY 1982 See the entire document	
A	US,A, 4,361,544 (GOLDENBERG) 30 NOV. 1982 See the entire document	
X	AU,B, 86 330/82 (GRIES ET AL) 22 JULY 1982 See the entire document	1-6
X — Y	EP,B, O 133 603 (CONIT ET AL) 27 FEB. 1985 , See the entire document	1-6,11-14,19 7-9,15-17,20, 21,26

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ²	Date of Mailing of this International Search Report ²
25 JUNE 1986	**2 3 JUL 1986**
International Searching Authority ¹	Signature of Authorized Officer ²⁰
ISA/US	Jerome R. Smith, Jr.

Form PCT/ISA/210 (second sheet) (October 1981)

	FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET	
X	FEBS LETTERS, VOLUME 168, NUMBER 1, ISSUED	1-6,11,12,14
Y	MARCH 1984. CHEN ET AL. "PARAMAGNETIC METALLOPORPHYRINS AS POTENTIAL CONTRAST AGENTS IN NMR IMAGING pp. 70-74. SEE TH ENTIRE DOCUMENT	7,8,13,15-17, 26
Y	AJR, MARCH 1984, WEINMANN ET AL, "CHARACTERISTICS OF GADOLINIUM-DTPA COMPLEX: A POTENTIAL NMR CONTRAST AGENT," pp. 619-624 SEE THE ENTIRE DOCUMENT	1-26

V.☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.☐ Claim numbers............, because they relate to subject matter [12] not required to be searched by this Authority, namely:

2.☐ Claim numbers............, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI.☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1.☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4.☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2)) (October 1981)